

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/SE05/000220

International filing date: 17 February 2005 (17.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: SE  
Number: 0400355-4  
Filing date: 17 February 2004 (17.02.2004)

Date of receipt at the International Bureau: 08 March 2005 (08.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

**PRV**PATENT- OCH REGISTRERINGSVERKET  
Patentavdelningen**Intyg  
Certificate**

Härmed intygas att bifogade kopior överensstämmer med de handlingar som ursprungligen ingivits till Patent- och registreringsverket i nedannämnda ansökan.

This is to certify that the annexed is a true copy of the documents as originally filed with the Patent- and Registration Office in connection with the following patent application.



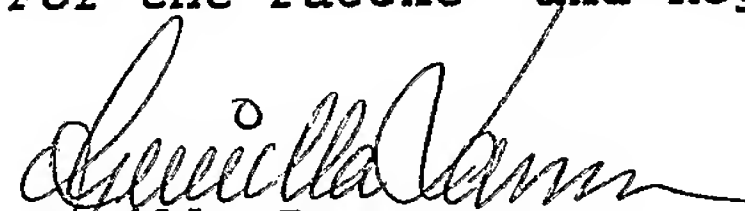
(71) Sökande                      Synbiotics AB, Stockholm SE  
Applicant (s)

(21) Patentansökningsnummer    0400355-4  
Patent application number

(86) Ingivningsdatum              2004-02-17  
Date of filing

Stockholm, 2005-02-21

För Patent- och registreringsverket  
For the Patent- and Registration Office

  
Gunilla Larsson

Avgift  
Fee

SÖKANDE:

SYNBIOTIC AB

UPPFINNINGENS BENÄMNING: NEW SYNBIOTIC USE

5

Field of Invention

The invention refers to a new use of a synbiotic composition and the organisms therein to prevent disease. More specifically, the invention refers to a new use of a composition, which comprises four lactic acid bacteria strains, for the manufacturing of a remedy for preventing and treating a stress induced inflammation disorder.

10

Background of the Invention

15

The morbidity and mortality in chronic as well as acute diseases, especially in the so called severely sick patients or critically ill patients have, despite claimed progress in medico-pharmaceutical and surgical treatments remained unaffected or increased during the last few decades. Much support that modern pharmaceuticals this far has been largely unable to stem the tide of these conditions. Especially the incidence of chronic diseases is fast increasing in the developed and but increasingly also in the developing world. A recent statistics from the World Health Organization (WHO) suggests that chronic diseases constitutes 46 % of global disease burden and 59 % of global deaths; each year on earth app 35 million people will die in chronic diseases. The epidemic of chronic diseases is closely associated with Western life style; physical and mental stress, reduced physical activity and consumption of refined, calorie-condensed fatty, sugary and starchy foods in combination with reduced intake of fibre- and antioxidant-rich fresh fruits and vegetables, but also bacteria. The secondary morbidity and mortality in association with advanced surgical and medical treatments, and in medical and surgical emergencies (physical injuries and acute diseases), already unacceptably high is also

20

25

30

35

increasing at a worrying rate, and affects mainly those with an incipient or manifest chronic disease. Sepsis is the most common complication both to advanced medical and surgical treatments. It is estimated that annually only in  
5 the US as much as 751 000 are affected by severe sepsis, leading to death in 215 000 patients (29%), and making sepsis the tenth most common cause of death (see Hoyert DL, Arias E, Smith BL et al. *National Vital Statistics Reports* (serial on line) [www.cdc.gov/nchs/data/nvsr/nvsr49/nvsr49](http://www.cdc.gov/nchs/data/nvsr/nvsr49/nvsr4908pdf)  
10 08pdf, and Angus DC, Linde-Zwirble WT, Lidicker J et al. (2001) *Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome and associated costs of care. Crit Care Med* 29:1303-1310).

Most worrying, the incidence Intensive Therapy Unit  
15 (ITU) associated sepsis seems to be rising with app 1.5 % per year, which most likely reflects a decreasing resistance to disease in the Society, but also the ineffectiveness of prophylactic and preventive measures, including prophylactic antibiotics. More than half of these patients  
20 (51 %) or 383 000 are treated in ITUs and another 130 000 ventilated in intermediary type care units. Medical emergencies like myocardial infarction, stroke and severe pancreatitis, originally not affected by sepsis, and extensive surgical and medical treatments in non-infectious  
25 conditions such as neurosurgical procedures and coronary by-pass surgery are also associated with unacceptably high 30-day septic morbidity. As deaths in such patients often are attributed to the original condition, the sepsis rate is in reality considerably higher than officially reported.  
30 The treatment costs per case is \$22,100 and the annual cost for the American nation to be at the level of about \$17 billions (Vincent J-L, Abraham E, Annane D et al. (2002). *Reducing mortality in sepsis: new directions. Critical Care* 6(Suppl 3):S1-S18). There are yet no health statistics  
35 common for the whole European Union, but most likely in

excess of one million patients will annually suffer from severe sepsis and more than 300 000 die in sepsis-associated conditions. Presently available treatment options, often very expensive to buy, as antibiotics and even more so antagonists or inhibitors of individual pro-inflammatory cytokines seem not as yet to offer any greater hope. Several decades of efforts to prevent and combat sepsis by various combinations of antibiotics and >30 randomised clinical trials have finally convinced us that vigorous antibiotic treatments such as selective decontamination of the digestive tract (SDD) does not significantly reduce mortality in critically ill patients.

Central to the development of chronic diseases is the occurrence of a condition called metabolic syndrome (characterized by obesity, hypertension, insulin resistance, glucose intolerance and fatty infiltration of almost all organs particularly the liver. Metabolic syndrome is a pre-stage of chronic diseases and a condition which today affects about 25 % of the population in countries like the US and the UK, and another 25 to 50 % are borderline. Much support that this condition is associated with over-consumption of calorie-condensed refined agricultural products and under-consumption of less calorie-condensed fruits, vegetables and bacteria. It should be noted that our Paleolithic forefathers consumed up to ten times as much plant fibres and billion times more of beneficial non-pathogenic microorganism than modern man.

The preventive flora is known to be significantly impaired in people living in Western countries and especially in individuals suffering from disease. As an example, 75 % of Americans have lost their flora of *Lactobacillus plantarum*, the lactic acid bacterium being the most frequent in the gut of individuals living a rural lifestyle. Our studies suggest that a majority of patients treated in hospital have a reduced flora, particularly

those treated in Intensive Care Units, who often have lost their entire lactobacillus flora. Our studies suggest that strong and bioactive flora can prevent or retard the development of chronic diseases, improve the condition for those suffering chronic diseases and reduce the infection rate following injuries, advanced medical and surgical treatments (operations).

Various producers of various probiotics do often claim health benefits from supplying different probiotics. In most cases this is unsubstantiated and not true. The reality is that only a small minority of the lactic acid bacteria have the health potentials requested. Most of the probiotics on the market, do not survive the acidity of the stomach, or the bile acid content of the small intestine, nor do they adhere to colonic mucosa and even temporary colonize the stomach. And no health benefits can be demonstrated. This was recently demonstrated when a standard commercial product, TREVIST™ (Chr Hansen Biosystem, Denmark) was tried in severely sick patients (Jain PK et al. *Influence of synbiotic containing Lactobacillus acidophilus LA5, Bifidobacterium lactis BP12, Streptococcus thermophilus, Lactobacillus bulgaricus and oligofructose on gut barrier function and sepsis in critically ill patients: a randomized controlled trial. Clinical Nutrition under publication*). Although significant reductions in number of potentially pathogenic organisms (PPMs) could be observed in the content of the stomach of the treated patients, no influence on intestinal permeability could be demonstrated nor any clinical benefits be demonstrated, when this particular formula was supplied to a mixed group of critically ill patients.

#### The Invention

In the present invention the lactic acid bacteria strains used are utilized with reference to the several



important functions that the natural flora in the body should have: to reduce/eliminate potentially pathogenic micro-organisms (PPMs), to reduce/eliminate the content in the gut of various toxins, mutagens/carcinogens, and to promote apoptosis. In combination with plant fibres used in the invention they should release from the fibres numerous nutrients, antioxidants, growth-, coagulation and other factors and to modulate innate and eventually also adaptive immune defence mechanisms. Such fibres from fruits and vegetables have strong bioactivities of their own: to maintain mucosal growth and functions, to maintain water and electrolyte balance, to provide energy and nutrients for the host, to provide energy and nutrients for the flora and to provide resistance against invading pathogens. The fermentation process in the lower GI tract releases through action of microbial enzymes numerous nutrients and antioxidants, but also various growth and coagulation-controlling as well as inflammation-controlling molecules. Antioxidants released in the lower GI tract have significant pro-regenerative, antibacterial, antithrombotic, vasodilatory, anti-inflammatory and anti-carcinogenic effects. Nitric oxide is also released by fermentation. It is known to control the pathogenic flora, to improve vascular circulation and intestinal motility. It also has dilatory effects on the airways.

The invention relates to a new use of a composition comprising four specifically selected bioactive lactic acid bacteria (LAB) The lactic acid bacteria are *Pediococcus pentosaceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *Lactobacillus paracasei* subsp *paracasei* 19, and *Lactobacillus plantarum* 2362. The lactic acid bacteria to be used were selected after extensive studies of > 350 human fecal bacteria and about 180 bacteria harvested from fresh growing rye. After extensive studies of numerous functions of each lactic acid bacterium were the four

lactic acid bacteria in the composition selected because of their unique and superior abilities: to survive in the low pH of the stomach and in the high bile acid content of the small intestine, unique ability to attach to colonic mucosa and to temporary colonize the large intestine, high capacity to ferment various types of plant fibres including rather fermentation resistant fibres such as inulin, a balanced production of both pro- and anti-inflammatory molecules such as cytokines, strong ability to produce several bioactive molecules, especially heat shock proteins and their production of significant amounts of antioxidants.

Accordingly, four specifically selected polymeric plant carbohydrates were included in the composition, beta-glucan, inulin, pectin and resistant starch. The four lactic acid bacteria and the four fibres in the composition are chosen for their documented unique and strong bioactivities and for synergistic functions in the body. When supplied in combination, the components of this synbiotic composition show strong synergistic/potentiated health benefits both in experimental animals and in humans. A significant increase in these functions was observed when the chosen fibres were included in the composition. The composition has been given the name Synbiotic 2000®.

According to the invention, a composition is used, which comprises four specifically selected lactic acid bacteria strains and a pharmaceutically acceptable liquid component, for the manufacturing of a remedy for preventing and treating a stress induced inflammation disorder. The strains used are *Pediococcus pentosaceus* 16:1, *Leuconostoc mesenteroides* 23-77:1, *Lactobacillus paracasei* subsp *paracasei* F-19, and *Lactobacillus plantarum* 2362.

The remedy is intended for human as well as veterinary use.



The bacterial strains used have been deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the Belgian Coordinated Collection of Microorganisms (BCCM), Gent, Belgium, under Accession No. LMG P-20608 for *Pediococcus penosaceus* 16:1, Accession No. LMG P-20607 for *Leuconostoc mesenteroides* 23-77:1, Accession No. LMG P-17806 for *Lactobacillus paracasei* (paracasei) F-19, and Accession No. LMG P-20606 for *Lactobacillus plantarum* 2362, also sometimes referred to as *Lactobacillus plantarum* 2592.

A concentrated lactic acid bacteria composition is diluted in dependence on the intended application. The four lactic acid bacteria strains are preferably administered in the pharmaceutically acceptable liquid component, in which they each has a concentration between about  $10^6$  and about  $10^{11}$  CFU/ml, preferably between about  $10^9$  and about  $10^{11}$  CFU/ml.

Such pharmaceutically acceptable liquid components are well known to those skilled in the art. Preferably, distilled water or buffered aqueous media are used, which contain pharmaceutically acceptable salts and buffers. Suitable salt solutions are PBS, PBSS, GBSS, EBSS, HBSS, and SBF. The liquid component can also be of a more hydrophobic nature in dependence of the application.

A suspension with the four lactic acid bacteria strains in the liquid component can then be inhaled.

Stress induced inflammation disorders, which can be prevented and treated by means of inhalation, are for example chest infections, such as those caused by cystic fibrosis. Pulmonary diseases can also be subjected to inhalation administration.

More evident effects are obtained by including fibers in the composition. Pronounced synergistic effects when

preventing and treating stress induced inflammation disorders are obtained by administering the lactic acid bacteria with the four polymeric carbohydrates, beta-glucan, inulin, pectin, and resistant starch. These components can each be administered in a dose of 1 to 10 g in the pharmaceutically acceptable liquid component.

By utilizing the gelling and thickening properties of both pectin and inulin, the composition comprising lactic acid bacteria and fibres can be administered as a gel. Such a gel can be used to prevent and treat such stress induced inflammation disorders as urinary tract infections and vaginal infections. Stress induced inflammations originating from burned surfaces can also be prevented and treated in this way, the burns also being electrical and chemical burns.

The major administration route for preventing and treating stress induced inflammation disorders is by oral ingestion of the synbiotic composition.

One third of Western people complain of GI problems, and inflammatory diseases affecting the intestine is an increasing burden. The synbiotic composition can with advantage be used when the stress induced inflammation disorder is an inflammatory bowel disease, e.g. for healing of colitis. It is also effective when the inflammatory bowel disease is a *Helicobacter* infection.

The synbiotic composition can with advantage also be used for preventing and treating postoperative disorders. Such postoperative disorders are for example those caused by postoperative infections. In this connection immunomodulatory effects have been obtained with the composition. The ability of the lactic acid bacteria to produce immunomodulins is an important property. The produced immunomodulins down-regulate the proinflammatory effect of proinflammatory cytokines, such as TNF.

The rate of infections is very high in transplant patients; for patients on waiting list for operation, in connection with the surgical procedure and during many years following replacement of the liver. The infection rate has despite all efforts to reduce it remained unaffected or eventually also increased in recent decades. Antibiotics seem to offer no prevention especially for bacterial infections. The 30-day infection rate after liver transplantation is usually reported to be above 50 %; the most recent study reports a 30-day morbidity of 86 % despite, or eventually due to extensive simultaneous treatment with four or more antibiotics, a treatment called selective bowel decontamination.

Accordingly, the composition can with advantage be used for controlling infections in transplantations, especially when the postoperative infection is an infection after liver transplantation.

Likewise, the synbiotic composition can be used with good results when the postoperative infection is a complication to advanced surgical operations or medical treatments, when the postoperative infection is an infection associated with acute critical illness, as well as when the postoperative infection is caused by supply of antibiotics and/or care in an Intensive Therapy Unit (ITU).

For example, abdominal cancer patients are susceptible to postoperative infections. Microbial overgrowth in the intestine is a major source of bacterial infection. Efforts to reduce the infection rate by aggressive antibiotic policies have generally failed. In this case, significant improvements in the levels of prealbumin, C-reactive protein, serum cholesterol, white cell blood count and serum endotoxin are obtained after administration of the synbiotic composition.

Likewise, when the postoperative infection is an infection after bone marrow transplantation, a faster

normalization of lymphocyte counts is obtained during the treatment.

Due to disease and the treatment with various pharmaceuticals the normal flora is in most patients severely deranged; the preventive flora is decreased and a significant overgrowth of potentially pathogenic microorganisms has occurred. Use of the synbiotic composition effectively eliminate potentially pathogenic microorganisms, restores the flora and reduces infections in ITU patients, as well as reduces postoperative infections.

Furthermore, the pathogenic organisms can be translocated into the whole body and develop more or less generalized sepsis. It is thus of importance that the synbiotic disorder can be used to prevent and treat septic disorders.

Frequently, the septic disorder is a chronic disease. Clinical improvements are obtained in patients with chronic diseases, such as chronic liver disease, who have been treated with the synbiotic composition. A reduction of liver enzymes is obtained. The intestinal levels of endotoxin-containing Gram-negative bacteria are reduced, which improves the systemic haemodynamic disturbance, especially in patients with liver cirrhosis. Improvements in bilirubin and prothrombin activity as well as in albumin level are also obtained.

Such a supply of the synbiotic composition to patients with advanced chronic liver disease is well-tolerated and no adverse events or changes in general clinical state have been observed. This observations is important as the means to long-term modulate the negative effects of proinflammatory cytokines on organs such as the liver are limited, very expensive and in addition full of severe side effects.

Thus, the use of a composition of four lactic acid bacteria strains and four polymeric carbohydrates can offer

an inexpensive and powerful tool with no side effects, and the composition can be used for long-term treatment of patients with liver disease.

Similar results are obtained when the chronic disease is chronic renal disease.

Other chronic diseases, which can be treated with the synbiotic composition, are chronic lung disease and cystic fibrosis.

Surprisingly, the synbiotic composition can prevent adhesion formations. By using the composition according to the invention the reoccurrence of adhesions after adhesiolysis can be limited and recurrence prevented.

Similarly, the lactic acid bacteria therein stimulate fibrinolytic activities and the composition can thus be used for preventing and treating thrombosis. Up to one third of operated patients have signs of occult thrombosis and the large majority of Western patients develop adhesions after surgery. Adhesions cause large problems as they are responsible for half of the cases of both intestinal obstructions and female infertility. In addition to increasing the costs of Society they cause extensive suffering and reoperations are difficult. It was estimated in 1995 that almost 300 000 adhesion operations were performed only in the US each year and that almost one million hospital days were used each year for treatment of adhesions. The annual costs for treatment of female infertility was estimated to about 1.2 billion dollars. It has been observed that germ-free animals develop no or almost no adhesions. Experimental evidence suggests that both adhesion formation and thrombosis development are strongly associated with an overexuberant superinflammation, which to a large extent originates in the gut.

Accordingly, a synbiotic composition comprising at least two specifically selected lactic acid bacteria strains and at least two specifically selected polymeric



carbohydrates can especially be used for:

- eliminating potentially pathogenic microorganisms;
- restoring the flora in ITU patients;
- reducing postoperative infections;
- 5 - reducing infections in ITU patients;
- controlling infections in transplantation;
- reducing serum endotoxin levels;
- reducing serum liver enzyme levels;
- controlling the production of heat shock proteins;
- 10 - increasing the production of antioxidants; and
- controlling the production of pro- and anti-inflammatory cytokines.

Preferably, the specifically selected lactic acid bacteria strains are *Pediococcus pentosaceus* 5-33:3, *Leuconostoc*  
15 *mesenteroides* 32-77:1, *Lactobacillus paracasei* subsp  
*paracasei* 19, and *Lactobacillus plantarum* 2362, and the specifically selected polymeric carbohydrates are beta-glucan, inulin, pectin and resistant starch.

## 20 EXAMPLES

The following illustrative examples show the unique documented effects obtained by using composition according to the invention.

### 25 Example 1. Effect in Intensive Therapy (ITU) patients.

A study was performed in 10 ITU patients. Samples were taken for bacterial cultivation from 10 patients. After anaerobic incubation for 48 hours at 37° C presence  
30 of LAB was attempted using API 50CHL identification strips (bioMerieux). In seven of the ten patients no growth of LAB could be detected.

Supplementing with the composition of four lactic acid bacteria strains and four polymeric carbohydrates for  
35 three days returned the LAB level in feces to the level about 10<sup>7</sup>. 18 patients in ITU received the composition and



were compared to 18 patients receiving placebo. The infection rate and mortality was less than half in the treated patients.

5 Example 2. Effect on development of adhesions and thrombosis.

A study was performed in thirty-six Wistar albino rats were divided into three groups, all subjected to a standard peritoneal adhesion model. During 3 weeks before  
10 the production of adhesions were the animals supplied by gavage with the composition of four lactic acid bacteria strains and four polymeric carbohydrates and a placebo consisting of the four polymeric carbohydrates, respectively. Seven days following the induction of adhesions the  
15 numbers and extent of adhesions were evaluated as well as two parameters of fibrinolysis: tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor type-1 (PAI-1) measured on biopsies from undamaged parietal peritoneum.

20 The adhesion score in the treated group was  $1.6 \pm 0.60$  compared to  $3.55 \pm 3.07$  in the control ( $P=0.0001$ ) The mean tPA level in the treatment groups was  $3.65 \pm 0.88$  compared to  $5.12 \pm 1.88$  in the control group ( $P=0.007$ ) and the PAI-1 in the treated group  $24.93 \pm 7.67$  compared to  $18.23 \pm 4.41$  in the  
25 control group (3  $P=0.004$ ).

When in an additional similar study extensive antibiotic treatment was tried, no similar benefits were obtained.

30 Example 3. Effect in abdominal cancer operations.

A prospective randomized double-blind trial was undertaken in 30 patients undergoing abdominal cancer operations. All patients received enteral nutrition with supply of a composition of four lactic acid bacteria  
35 strains and four polymeric carbohydrates. A comparison was

made between one group receiving the composition and another group receiving a standard clinical nutrition.

The incidence of postoperative bacterial infections during the first month after operation was 6.7 % compared to 47 % in the control. Laboratory studies performed on days 3 and 6 after the operation demonstrated significant improvement parameters such as prealbumin, C-reactive protein, serum cholesterol, white cell blood count and serum endotoxin. The first defecation occurred significantly earlier in the group that had been treated with the composition according to the invention.

Example 4. Effect in transplant patients.

A prospective randomized double-blind trial was undertaken in 66 liver transplant recipients. A comparison was made between one group receiving the synbiotic composition of four lactic acid bacteria strains and four polymeric carbohydrates and a group receiving only the four bioactive polymeric carbohydrates therein. The treatment started the day before surgery and continued for 14 days.

The incidence of postoperative bacterial infections was significantly reduced from 48% with only fibres to only 3% (one of 33 patients developed a slight urinary infection). Bacterial cultures showed microbial growth in 8 of the treated patients and 16 of the control patients. The most frequently identified microorganisms were gut-derived, with a predominance of *Enterococcus faecalis* and *faecium* observed.

In addition, three patients undergoing bone marrow transplantation have been treated with the composition according to the invention. No episodes of infection was observed. The in-hospital time was record short. In addition it was observed that the lymphocyte counts normalized quicker than normally seen.

Example 5. Effect on inflammation and infection in chronic liver disease.

Western people, especially those who are obese and suffer metabolic syndrome, have also fatty livers. Fatty livers is especially seen in persons with visceral obesity. Visceral fat cells (adipocytes) have a much increased expression of cytokines, especially of TNF $\alpha$ . The amount of fat in the abdomen can vary from a few milliliters to about six liters, which can explain the increased exposure of proinflammatory molecules, such as TNF $\alpha$ , in adipose individuals. Increased release of proinflammatory molecules, such as TNF $\alpha$ , together with an over-expression of  $\gamma$ -interferon and underexpression of IL-10 sensitizes the liver to both endotoxins and to toxic effects, especially of TNF $\alpha$ , which most likely is a key factor behind the progressive liver damage seen in patients with liver cirrhosis.

The supply of the four lactic acid bacteria strains (probiotics) alone or in combination with the four polymeric carbohydrates (synbiotics) have the ability not only to reduce the production and absorption of endotoxin in the intestine, but also to down-regulate the production of proinflammatory cytokines, including TNF $\alpha$ . Furthermore, the composition induces in patients with liver disease significant improvements in bilirubin and prothrombin activity as well as in albumin level.

A long-term supply of a composition of four lactic acid bacteria strains and four polymeric carbohydrates reduces both the inflammation of the liver, the fatty infiltration of the liver (steatosis) and retards the progress of liver destruction.

When the synbiotic composition of four lactic acid bacteria strains and four polymeric carbohydrates was supplied to eleven patients with chronic liver disease a significant reduction in production of TNF $\alpha$  by peripheral

blood mononuclear cells in response to stimulation by endotoxin or *Staphylococcus aureus* enterotoxin B (SEB), was reduced to half in comparison to pre-supplementation levels in the majority of cirrhotic patients, who were supplied with the composition.

Example 6. Effect on gut colonisation, liver function and degree of encephalopathy in chronic liver disease.

The synbiotic composition of four lactic acid bacteria strains and four polymeric carbohydrates was supplied during one month to patients with chronic liver disease and results compared to 15 similar patients, who received a placebo (non-fermentable, non-absorbable fibre) during the same time period.

The intestinal pH was significantly reduced in the treatment group compared to placebo. Significant decreases in the number of *Escherichia coli*, *Staphylococcus* and *Fusobacterium* were observed. Significant decreases in ammonia(s), levels of endotoxin(s) and ALT (expression of impaired liver function) were observed in the group receiving the synbiotic composition, but not in the placebo group. The improvements in liver function observed in the group supplemented the synbiotic composition were accompanied by significant improvements also in psychometric tests and in degree of encephalopathy.

Example 7. Effect on liver blood flow and indocyanine clearance.

Indocyanine clearance (ICG<sub>R15</sub>) test, a measurement of liver blood flow, was performed in 15 patients.

A supplementation with the synbiotic composition of four lactic acid bacteria strains and four polymeric carbohydrates resulted in a significant reduction in ICG<sub>R15</sub> in the cirrhotic patients, an improvement most likely due to a

reduced swelling of endothelial and sinusoidal cells and hereby reduced resistance to flow.

Accordingly, a long-term supplementation of the synbiotic composition has the potential to reduce the number and the degree of severity of bleeding episodes in patients with liver cirrhosis.

Example 8. Effect in colitis patients.

Ten patients with moderate colitis were treated during two weeks with twice daily enemas of the synbiotic composition reconstituted in 120 ml normal saline. Studies were repeated on days 0, 7, 14 and 21. Significant reduction in diarrhoea scores were observed at days 7, 14, and 21. Visible blood in stool was significantly reduced at days 14 and 21. Also nocturnal diarrhoea, urgency to defecation and consistency of stool were significantly reduced at days 7, 14, and 21.

A stronger effect obtained with the four specific lactic acid bacteria strains, especially when combined with the four specific polymeric carbohydrates.

Example 9. Effect on Helicobacter colonization of the stomach.

Ten animals and six humans with *Helicobacter* infection in the stomach received the composition of four lactic acid bacteria strains and four polymeric carbohydrates for 14 days. A total elimination of *Helicobacter* colonies was observed.

Example 10. Effects on chest infections.

Several patients with cystic fibrosis have been treated with the composition of four lactic acid bacteria strains and four polymeric carbohydrates. There have all been in a rather late stage of disease, losing weight, suffering diarrhea, and on almost constant antibiotic treatment. They have all made a dramatic turn around,

diarrhea has been controlled, gained weight, and need of antibiotics been eliminated.

Example 11. Effects when applied topically.

5           Thirty-five patients with infected burns on skin were  
treated with a gel of the synbiotic composition of four  
lactic acid bacteria strains and four polymeric carbo-  
hydrates. A dramatic reduction of infection and cleaning  
of the burned surfaces was observed. An improved healing  
10       could also be noted.

Five patients with vaginitis were treated with a gel  
of the composition of four lactic acid bacteria strains and  
four polymeric carbohydrates. An instant cure was observed.

15           Eleven patients with infections around entrances  
of foreign material through the skin (trachostomies,  
infusion lines, drainages) were treated topically with  
a gel of the composition of four lactic acid bacteria  
strains and four polymeric carbohydrates. A quick eli-  
mination of the biofilm and cleaning of the skin around  
20       the foreign material was observed.

Example 12. Effects when inhaled.

A significant increase in ventilary volume was  
observed in 8 experimental animals on inhaling the four  
25       lactic acid bacteria strains dissolved in saline as a  
composition.

Accordingly, the composition can be used for cleaning  
of mucosal linings in the lungs, and for the dilatation of  
the air ways.

30



## CLAIMS

1. Use of a composition, which comprises four lactic acid bacteria strains, *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and a pharmaceutically acceptable liquid component, for the manufacturing of a remedy for preventing and treating a stress induced inflammation disorder.

2. Use as in claim 1, wherein said four lactic acid bacteria strains in said pharmaceutically acceptable liquid component each has a concentration of  $10^6$ - $10^{11}$  CFU/ml.

3. Use as in claim 1, wherein said composition is to be inhaled.

4. Use as in claim 3, wherein said stress induced inflammation disorder is a chest infection.

5. Use as in claim 4, wherein said chest infection is caused by cystic fibrosis.

6. Use as in claim 3, wherein said stress induced inflammation disorder is a pulmonary disease.

7. Use as in claim 1, which composition further comprises four polymeric carbohydrates, beta-glucan, inulin, pectin, and resistant starch.

8. Use as in claim 7, wherein said four polymeric carbohydrates in said pharmaceutically acceptable liquid component each comprises 1-10 g/dose.

9. Use as in claim 7, wherein said composition is to be applied topically.

10. Use as in claim 9, wherein said composition is administered as a gel comprising said four polymeric carbohydrates.

11. Use as in claim 9, wherein said stress induced inflammation disorder is a urinary tract infection.

12. Use as in claim 9, wherein said stress induced inflammation is a vaginal infection.

13. Use as in claim 9, wherein said stress induced inflammation is a burned surface.

14. Use as in claim 7, wherein said composition is to be ingested.

5 15. Use as in claim 14, wherein said stress induced inflammation disorder is a postoperative disorder.

16. Use as in claim 15, wherein said postoperative disorder is adhesion formation.

10 17. Use as in claim 15, wherein said postoperative disorder is thrombosis.

18. Use as in claim 15, wherein said postoperative disorder is a postoperative infection.

19. Use as in claim 18, wherein said postoperative infection is an infection after liver transplantation.

15 20. Use as in claim 18, wherein said postoperative infection is an infection after bone marrow transplantation.

20 21. Use as in claim 18, wherein said postoperative infection is a complication to other advanced surgical operations or medical treatments.

22. Use as in claim 18, wherein said postoperative infection is an infection associated with acute critical illness.

25 23. Use as in claim 18, wherein said postoperative infection is caused by supply of antibiotics and/or care in an Intensive Therapy Unit.

24. Use as in claim 14, wherein said stress induced inflammation disorder is a septic disorder.

30 25. Use as in claim 24, wherein said septic disorder is a chronic disease.

26. Use as in claim 25, wherein said chronic disease is a chronic liver disease.

27. Use as in claim 25, wherein said chronic disease is chronic renal disease.

28. Use as in claim 25, wherein said chronic disease is chronic lung disease.

29. Use as in claim 25, wherein said chronic disease is cystic fibrosis.

5 30. Use as in claim 14, wherein said stress induced inflammation disorder is a systemic haemodynamic disturbance.

31. Use as in claim 14, wherein said stress induced inflammation disorder is an inflammatory bowel disease.

10 32. Use as in claim 31, wherein said inflammatory bowel disease is colitis.

33. Use as in claim 31, wherein said inflammatory bowel disease is a *Helicobacter* infection.

15 34. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for controlling the production of pro- and anti-inflammatory cytokines.

20 35. Use as in claim 34, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

25 36. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for controlling the production of heat shock proteins.

30 37. Use as in claim 36, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606),

and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

38. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for increasing the production of antioxidants.

39. Use as in claim 38, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

40. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for reducing serum endotoxin levels.

41. Use as in claim 40, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

42. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for reducing serum liver enzyme levels.

43. Use as in claim 42, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

44. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for eliminating potentially pathogenic microorganisms.

5       45. Use as in claim 44, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606),  
10 and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

46. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for  
15 restoring the flora in Intensive Therapy Unit patients.

47. Use as in claim 46, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606),  
20 and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

48. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for  
25 reducing infections in Intensive Therapy Unit patients.

49. Use as in claim 48, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606),  
30 and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.

50. Use of a composition, which comprises at least  
35 two lactic acid bacteria strains and at least two polymeric

carbohydrates, for the manufacturing of a remedy for reducing postoperative infections.

51. Use as in claim 50, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.
52. Use of a composition, which comprises at least two lactic acid bacteria strains and at least two polymeric carbohydrates, for the manufacturing of a remedy for controlling infections in transplantation.
53. Use as in claim 52, wherein said at least two lactic acid bacteria strains are *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and said at least two polymeric carbohydrates are beta-glucan, inulin, pectin, and resistant starch.



## ABSTRACT

The invention refers to the use of a composition for the manufacturing of a remedy for preventing and treating a stress induced inflammation disorder. The composition comprises four specifically selected lactic acid bacteria strains, *Pediococcus pentosaceus* 16:1 (LMG P-20608), *Leuconostoc mesenteroides* 23-77:1 (LMG P-20607), *Lactobacillus paracasei* subsp *paracasei* F-19 (LMG P-17806), and *Lactobacillus plantarum* 2362 (LMG P-20606), and a pharmaceutically acceptable liquid component. The composition may further comprise four specifically selected polymeric carbohydrates, beta-glucan, inulin, pectin, and resistant starch.

A composition comprising at least two specifically selected lactic acid bacteria strains and at least two specifically selected polymeric carbohydrates can further be used for eliminating potentially pathogenic microorganisms, restoring the flora in ITU patients, reducing postoperative infections, reducing infections in ITU patients, controlling infections in transplantation, reducing serum endotoxin levels; reducing serum liver enzyme levels, controlling the production of heat shock proteins, increasing the production of antioxidants, and controlling the production of pro- and anti-inflammatory cytokines.